EXHIBIT 41

TALC AND CARCINOMA OF THE OVARY AND CERVIX

BY

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Summary

An extraction-replication technique was used to examine tissue from patients with ovarian and cervical tumours. In both conditions talc particles were found deeply embedded within the tumour tissue. The close association of talc to the asbestos group of minerals is of interest.

THE development in this laboratory of an extraction-replication technique (Henderson, 1969) for the study of foreign particles within tissues has allowed the *in situ* identification of crocidolite asbestos within the tissue of various mesotheliomas (Henderson *et al.*, 1969) removed from patients who had been concerned with the manipulation of asbestos in industry. This technique has now been applied to the study of tissue from ovarian and cervical carcinoma.

MATERIALS AND METHODS

Tissue

The tissue studied was obtained from patients with cancer of either the ovary or the cervix, and was first prepared as paraffin sections for normal routine histological examination but was unstained. Sections were then stained for histological assessment in the usual manner, and adjacent unstained tissue prepared for electron microscopy.

Replication Technique

The extraction-replication procedure has been described (Henderson, 1969). Sections of tissue were immersed in xylene and in ethanol, and the dehydrated tissue was then embedded by impressing the section on to the surface of a thin sheet of acetone-softened cellulose acetate, mounted on a glass slide, and left to harden. On removing the slide, the embedded tissue was left in the cellulose acetate. The tissue was then outlined with thin strips of Scotch tape to form a shallow well, and a 10 per cent (v/v) polyvinyl alcohol (PVA) solution applied. When the PVA had hardened it was stripped from the section providing a replica of the tissue surface. Foreign particles associated with the tissue are often removed with the PVA during this stripping process.

A complete sequential examination through the embedded tissue is possible by taking successive strippings. These surface replicas were then preshadowed with platinum, a carbon film deposited for strength, and the PVA removed by floating the replica in a hot water bath. Replicas were mounted on electron microscope grids for examination, using the AEI-6B microscope.

RESULTS

No asbestos particles were found in any of the tissue studied. Particles of talc were identified in approximately 75 per cent (10 of 13) of the

FIG. 1
Typical decoration pattern on a particle of natural talc. Numerous crystal lattice planes are shown (a). (×30 000.) Scale refers to 1·0 μ.

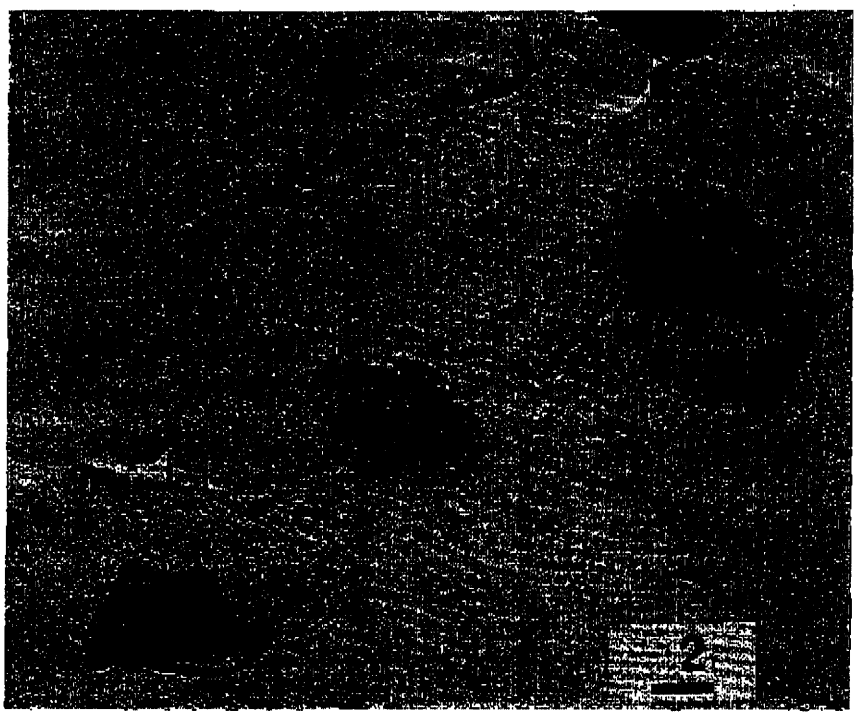

FIG. 2
Commercial talc preparations illustrating the decoration pattern. (×40 000.)

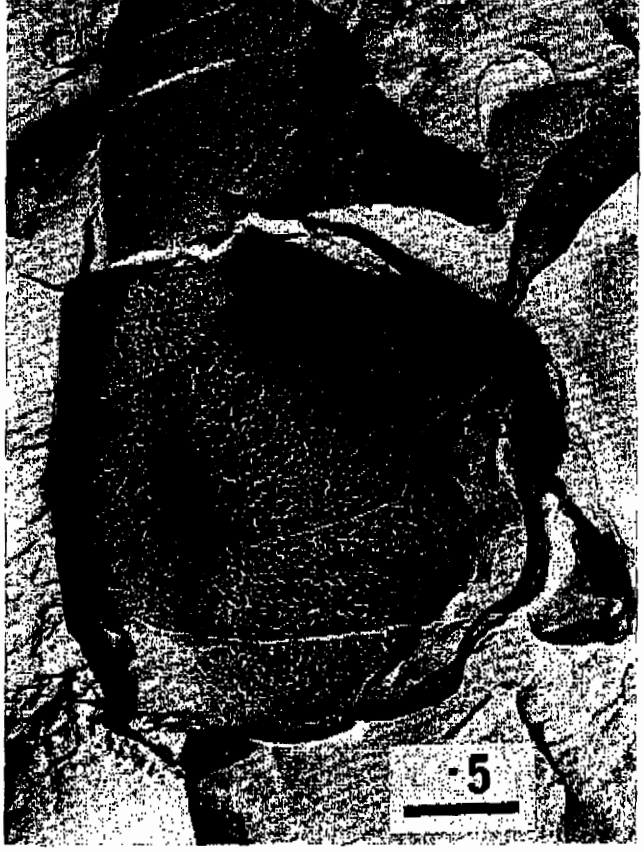

FIG. 3.

Micrograph of tissue from a serous papillary cystadeno-carcinoma of the ovary removed from a 27-year-old female. No previous abdominal operations had been carried out. The decoration pattern and lattice planes are shown. (×30 000.)

ovarian tumours. Using the replication technique identification of talc is possible because of the characteristic "decoration pattern" induced by the evaporation of platinum *in vacuo* on the crystal surface. Figure 1 shows this pattern on a particle of *natural* talc and the distinctive lattice planes of the crystals. Anthophyllite asbestos, which is known to be converted naturally to talc, is the only crystalline material which is at present indistinguishable from talc by using the replication technique. The decoration pattern on material from a commercial talc preparation is also demonstrated in Figure 2.

Material found within the ovarian tumours and identified as talc is illustrated in Figure 3. The talc particles were found deep within the tumour tissue. Some were as small as 1000Å in size but they were generally within a range from 1000Å to 2 μ.

Talc particles were also found embedded within tumours of the cervix. Figure 4 shows one such particle embedded in a capillary wall within the tumour, and Figure 5 illustrates the decoration pattern of the particle at a higher magnification. Crystals as large as 5 μ. were found in tissue from the cervical tumours and were generally larger than those seen in the ovarian tumours. Talc crystals were found in

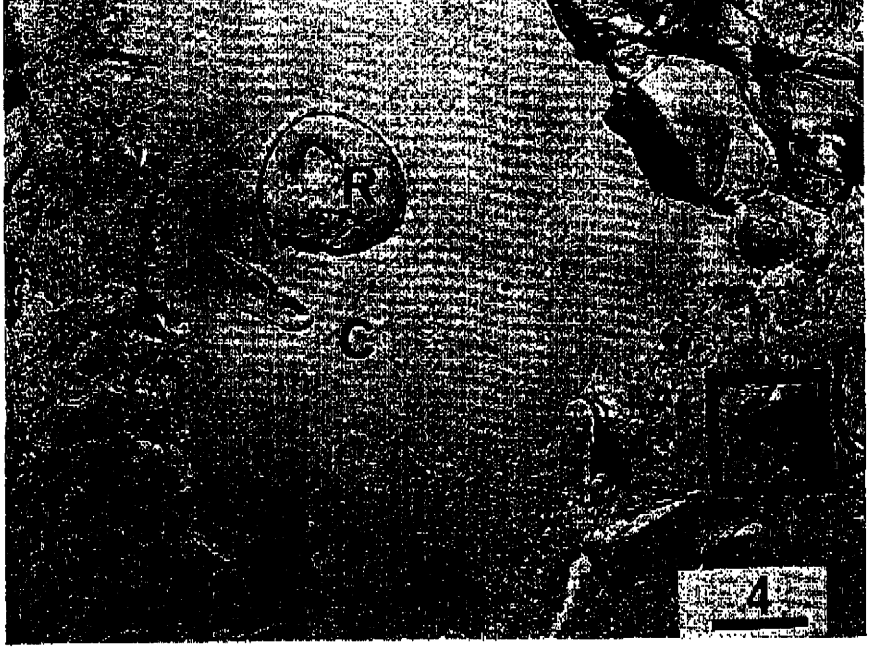

FIG. 4

Micrograph of tissue from a squamous-cell carcinoma of the cervix from a 62-year-old female. C—capillary, R—red cell. The particle of talc can be seen in the wall of the capillary. (×3500.)

Figure 3. within the ; 1000Å in ange from

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FIG. 5

A higher magnification of the talc particles outlined in Fig. 4. The typical decoration pattern is shown. (×40 000.)

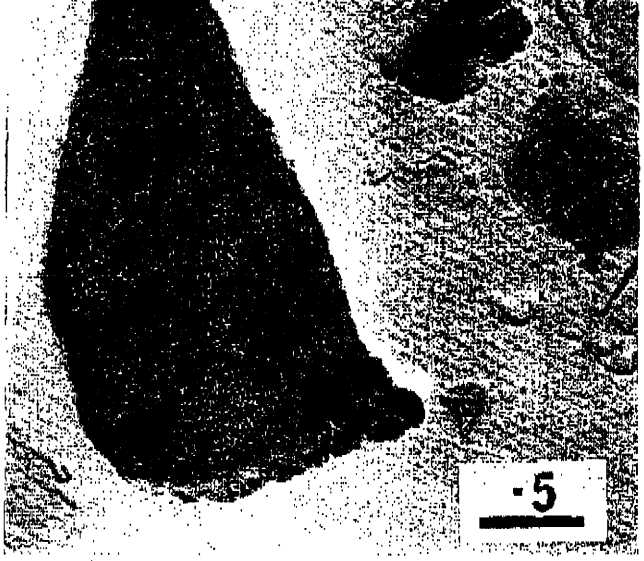

FIG. 6
Talc particles found in tissue from a pneumoconiotic lung. (×30 000.)

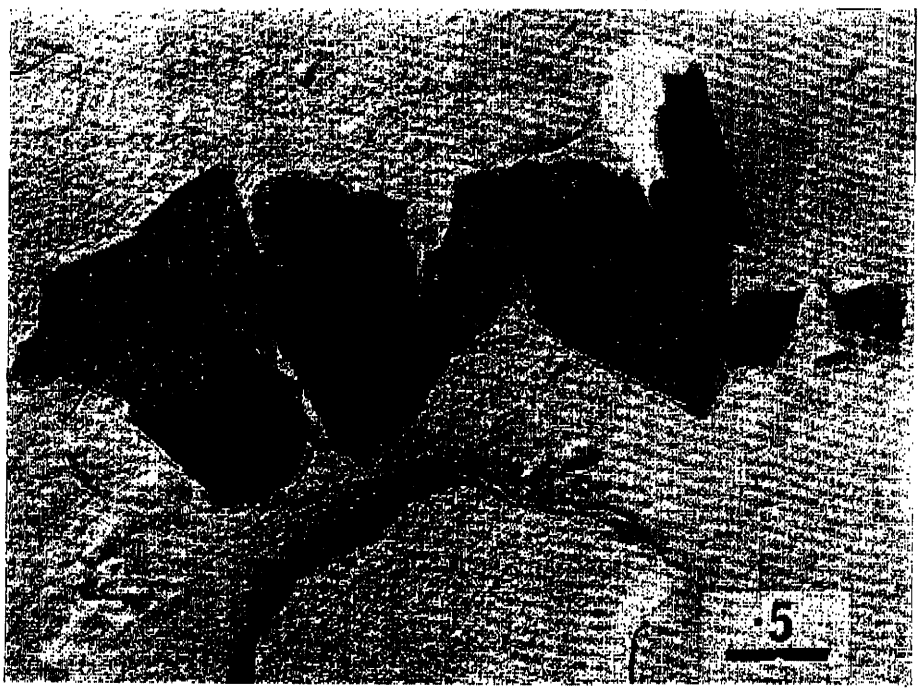

FIG. 7
Micrograph from the deepest part of an extensive papillary adenocarcinoma entirely replacing the endometrium in a 58-year-old woman, 8 years postmenopausal. Both ovaries were enlarged by hilar metastases, showing histological features similar to the primary endometrial lesion. Numerous talc particles were found in the primary endometrial carcinoma, but none in the metastatic ovarian tumours. (×26 000.)

minute, often with the dimensions of viruses, and only small regions of the tumour tissue could be studied. Approximately ten replication "strippings" for electron-microscope examination are usually taken from each thin section of the tissue. Figure 6 illustrates the use of the technique in the examination of pneumoconiotic lung tissue from a patient whose industrial history indicated long exposure to Norwegian talc.

Many particles of talc were found concentrated in the deeper layers of a primary carcinoma of the endometrium (Fig. 7) whereas extensive studies of a secondary tumour in the ovary in the same patient did not show the presence of talc. Application of the technique to "normal" ovarian tissue removed from patients with breast cancer has also shown talc particles in 5 of 12 such tissues studied. Extensive study at high magnification with the electron microscope is, however, required for evaluation of a replica and particles could easily be missed.

The application of electron-microscope microanalysis (EMMA-AEI, Harlow, England) to the particles extracted by the replication technique has provided preliminary evidence that the crystals contain magnesium and silicon, talc being a magnesium silicate.

Discussion

The possibility that the increasing incidence of carcinoma in western society may be related to a corresponding increase in the use of asbestos (Graham and Graham, 1967) is of interest, especially with regard to pleural and peritoneal mesotheliomas in workers exposed to crocidolite asbestos in industry (Wagner *et al.*, 1960; Elwood and Cochrane, 1964). There have been a number of reports about the relationship between asbestos and carcinogenesis (Smith *et al.*, 1965; Jacob and Anspach, 1965). However, the identification of asbestos fibres within tissue is extremely difficult. Fine particles embedded within tumour tissue are usually beyond the limits of resolution of the optical microscope, and tissue incineration, followed by electron microscopy of the isolated particles, may be unreliable if chemical changes are ferritin bodies on some of the fibres, although these cannot easily be distinguished from similar bodies around elastin fibres (Henderson *et al.*, 1970). This procedure may not, however, be as unreliable as the use of polarized light for the demonstration of brightly illuminated "birefringent crystals of asbestos".

The replication technique (Henderson, 1969) failed to show asbestos fibres in the ovarian neoplasms studied. On the other hand, there was good evidence for the presence of talc, often indistinguishable from anthophyllite asbestos, within the ovarian tissue. (Anthophyllite is converted naturally to talc.) The talc particles were found localized deep within tumour tissues, and not universally dispersed throughout the tumour. The talc particles in the ovary were generally much smaller than those found in the tissue from the tumours of the cervix.

The relationship between asbestos and mesotheliomas appears well established, and the replication technique has provided unequivocal evidence for the presence of fibres within such tumours. This technique has also produced evidence for the presence of talc in tissue from pneumoconiotic lungs of a patient with an industrial history of exposure to Norwegian talc (Henderson *et al.*, 1970). The presence of mica, kaolin and asbestos fibres were also identified in tissue from these pneumoconiotic lung tissue.

Although it is impossible to incriminate talc as a primary cause of carcinomatous changes within either the cervix or the ovary on the preliminary observations described here, the possibility that talc may be related to other predisposing factors should not be disregarded and further investigations are obviously required.

Acknowledgements

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